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Metabolism of atrial and brain natriuretic peptides in the fetoplacental circulation of fetuses with congenital heart diseases

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ABSTRACT

Introduction: Natriuretic peptides (NPs) play a pivotal role in maintaining fetal circulation; however, little is known about their metabolism. The aim of the present study was to elucidate the metabolism of plasma NPs in the fetoplacental circulation.
Methods: Plasma NP concentrations in maternal vein and umbilical artery (UA) and vein (UV) samples from fetuses with congenital heart defect (n = 86) or arrhythmia (n = 31) and controls (n = 127) were analyzed.
Results: Levels of plasma atrial NP (ANP) and brain NP (BNP) showed good correlation between UV versus UA samples ($p < 0.01$). In all three fetus groups, the regression coefficients between UV and UA plasma ANP levels were close to 0.5, while those between UV and UA plasma BNP levels were close to 1. The molecular forms of immunoreactive ANP in UA plasma showed a single peak corresponding to mature ANP, while those of immunoreactive BNP in UA plasma showed two major peaks and several minor peaks corresponding to mature BNP-32 and its partially digested peptides, as well as glycosylated and non-glycosylated BNP precursors (proBNP). No correlation was found between fetuses and mothers in terms of either plasma ANP or BNP levels.
Conclusions: The mother and fetus independently secrete and metabolize both ANP and BNP. Fetal plasma ANP consists exclusively of the mature form, and the placenta and umbilical vessels are possible major sites of ANP metabolism. In contrast, fetal plasma BNP consists predominantly of the precursor forms, which may contribute to protecting BNP from metabolism in the fetoplacental circulation.

1. Introduction

Natriuretic peptide (NP) is known to induce natriuresis, inhibit aldosterone secretion and reduce intravascular volume and pressure [1]. Integration of these actions regulates arterial pressure as well as cardiac filling pressure and output. Atrial NP (ANP) is mainly synthesized and stored in the atria, while brain NP (BNP) is mainly released from ventricular myocytes [2]. ANP is known to circulate as a mature alpha-ANP with full bioactivity, while BNP in the blood is mainly composed of mature fully active BNP-32 and its precursor proBNP [2]. Both ANP and BNP primarily function as endogenous ligands of NP receptor A (NPR-A), a guanylyl cyclase membrane receptor. Two pathways have been described for the clearance of circulating NPs: binding and internalization via NP receptor C (NPR-C), and enzymatic degradation by peptidases [3,4].

Since higher concentrations of ANP and BNP are detectable in fetal plasma than in adult plasma, NPs are assumed to undergo strict physiological regulation in fetal cardiovascular homeostasis and development [5]. Our previous study demonstrated that umbilical cord plasma NP levels reflect the severity of heart failure in fetuses with congenital heart defect (CHD) or arrhythmia [6]. As in adults, ANP and BNP constitute a dual NP system responsive to changes in cardiac filling

Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; bpm, beats per minute; cGMP, cyclic guanosine-3′,5′-monophosphate; CHD, congenital heart defect; glyco, glycosylated; NEP, neutral endopeptidase; NP, natriuretic peptide; NPR, natriuretic peptide receptor; pro brain natriuretic peptide, BNP precursor; RP-HPLC, reverse-phase high-performance liquid chromatography; RT-PCR, reverse transcription polymerase chain reaction; TFA, trifluoroacetic acid; UA, umbilical artery; UV, umbilical vein

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pressure [7]. In contrast, little is known about the metabolism and clearance of ANP and BNP in the fetoplacental circulation [5]. The placenta plays a pivotal role in maintaining fetal circulation, and is also presumed to metabolize peptide hormones [8]. NPs may help regulate the blood supply to the fetus, acting as vasodilators in the placental vasculature [9]. Therefore, in this study, we hypothesized that the placenta contributed to the metabolism of ANP and BNP in fetuses with CHD or arrhythmia.

The aim of the present study was to investigate the metabolism of ANP and BNP in the fetoplacental circulation of fetuses with CHD or arrhythmia. We cross-sectionally compared plasma NP concentrations in samples from the maternal vein and umbilical artery (UA) and vein (UV) at birth. We also investigated the molecular forms of ANP and BNP in the fetoplacental circulation.

2. Materials and methods

2.1. Study population

A single-center cross-sectional study was conducted with the approval of our institutional ethics board (M24-041). Written informed consent was obtained from the parents. This was a secondary analysis, and detailed inclusion and exclusion criteria were described in our previous report [6]. In brief, all singletons prenatally diagnosed with fetal CHD or arrhythmia at the National Cerebral and Cardiovascular Center of Japan between October 2012 and December 2015 were enrolled in this study. Exclusion criteria included critical chromosomal anomalies such as trisomy 13 or 18, and critical extracardiac anomalies that required surgical intervention during the neonatal period. Since extrasystoles are common in fetal life and generally have no consequence, they were excluded from the present study. Controls were consecutively recruited women with normal fetuses that had no complications such as CHD, extracardiac anomaly, and growth restriction. Maternal and obstetrical complications such as chronic hypertension, diabetes mellitus, preeclampsia, and gestational diabetes mellitus were also excluded from cases and controls.

2.2. Diagnosis and management of CHD and arrhythmia

In all fetuses, CHD was diagnosed prenatally using fetal echocardiography performed with Voluson E8 ultrasound equipment (GE Medical Systems, Zipf, Austria). Our tertiary pediatric cardiac center has an established protocol for patients with a prenatal diagnosis of fetal CHD or arrhythmia [6,10]. All diagnoses of CHD were confirmed soon after birth by pediatric cardiologists. All fetal arrhythmias were diagnosed using fetal echocardiography and magnetocardiography (MC-6400, Hitachi High-Technologies Corporation, Tokyo, Japan). Fetal arrhythmias were categorized as tachyarrhythmia or bradyarrhythmia, which were defined as a ventricular rate of ≥180 beats per minute (bpm) and < 100 bpm, respectively.

2.3. Sample collection and measurement

Maternal vein blood samples were obtained before delivery, while UA and UV blood samples were obtained at delivery. All blood samples were immediately collected in test tubes containing EDTA-2Na and aprotinin (final concentrations, 1.5 mg/mL and 500 kallikrein inhibitor units/mL, respectively). After blood samples were chilled on ice, plasma samples were obtained by centrifugation at 1500 × *g* for 15 min at 4 °C and stored at −80 °C until measurement. Plasma ANP and BNP concentrations were measured using the AIA-PACK chemiluminescence immunoassay (Tosoh Corporation, Tokyo, Japan).

2.4. Reverse-phase high-performance liquid chromatography (RP-HPLC) analysis

Each plasma sample was loaded onto a Sep-Pak C18 cartridge (Waters, Milford, MA, USA), pre-washed according to the manufacturer's instructions and pre-equilibrated with 0.9% NaCl. The cartridge was washed with 0.9% NaCl and 10% CH_3CN/0.1% trifluoroacetic acid (TFA), and then eluted with 60% CH_3CN/0.1% TFA. Eluates were subjected to RP-HPLC on a C18 column (Symmetry 300, 3.9 × 150 mm, Waters). RP-HPLC was performed using a linear gradient of CH_3CN from 10% to 60% in 0.1% TFA at a flowrate of 1.0 mL/min for 40 min. An aliquot of each fraction obtained by the RP-HPLC was evaporated and lyophilized, and one-fifth of each fraction was subjected to AIA-PACK chemiluminescence immunoassay (Tosoh) of ANP and BNP. The retention times of synthetic human ANP (alpha-ANP of 28 residues, Peptide Institute, Osaka, Japan), BNP (BNP-32 of 32 residues, Peptide Institute), proBNP, and glycosylated proBNP (HyTest, Turku, Finland) were confirmed by the RP-HPLC.

2.5. Reverse transcription polymerase chain reaction (RT-PCR) analysis

Gene expressions of *NPR1* and *NPR3* were determined with RT-PCR analysis of total RNA from placental tissue samples from two healthy women (Takara Bio Inc., Shiga, Japan and Agilent Technologies Ltd., CA, USA).

2.6. Statistical analysis

Statistical analysis was performed using JMP 11 (SAS Institute, Cary, NC, USA). Data are presented as means ± standard deviation or numbers of patients. Student's t-test or the Wilcoxon rank sum test was used to compare continuous variables between groups, as appropriate. Categorical variables were evaluated using Fisher's exact test. The Steel-Dwass test was used to compare continuous variables among three or more groups. Correlation analysis was performed using Pearson's coefficients. A value of $p < 0.05$ was considered significant in all analyses.

3. Results

3.1. Study cohort and baseline characteristics

A total of 143 fetuses with CHD or arrhythmia and 137 controls were prospectively enrolled in the present study [6]. In the CHD and arrhythmia groups, four cases of fetal demise, three cases of trisomy 18, seven cases of sampling failure, and 12 cases of extrasystole were excluded, leaving 117 fetuses available for analysis. Fetal demise was due to Ebstein's anomaly with circular shunt in two cases, dilated cardiomyopathy in one case, and a double-outlet right ventricle with severe fetal growth restriction in the final case. The distributions of CHDs (n = 86) and arrhythmias (n = 31) in this study are shown in Table 1. Since we already showed that morphological abnormality of the heart is not a primary perinatal factor associated with high NP levels in umbilical cord blood [6], arrhythmias complicated by CHD were classified as part of the arrhythmia group. One fetus with supraventricular tachycardia had a cardiac tumor, and two fetuses with complete atrioventricular block and two with sinus bradycardia had left atrial isomerism. Fetal therapy was performed in 22 fetuses with the following arrhythmias: supraventricular tachycardia or atrial flutter (n = 15), ventricular tachycardia (n = 2), and complete atrioventricular block (n = 5). In the control group, one case with fetal hydronephrosis and nine cases with sampling failure were excluded, leaving 127 fetuses available for analysis. Baseline perinatal characteristics are shown in Table 2. All controls had normal fetal growth and a CVP score of 10. Cesarean delivery was generally performed in the control group due to previous cesarean delivery. Maternal plasma ANP and BNP levels were

Table 1
Categories of CHD and/or arrhythmia (n = 117).

CHD (n = 86)	
Isomerism (n = 15)	Right atrial isomerism (n = 12)
	Left atrial isomerism (n = 3)
Hypoplastic left heart syndrome (n = 6)	
Right heart defect (n = 20)	Ebstein's anomaly or tricuspid valve dysplasia (n = 6)
	Pulmonary atresia with an intact ventricular septum (n = 4)
	Tricuspid atresia (n = 10)
Cyanotic heart defect (n = 28)	Transposition of the great arteries (n = 7)
	Double outlet right ventricle (n = 5)
	Tetralogy of Fallot (n = 12)
	Truncus arteriosus (n = 4)
Acyanotic heart defect (n = 17)	Coarctation of the aorta (n = 9)
	Atrioventricular septal defect (n = 8)
Arrhythmia (n = 31)*	
Tachyarrhythmia (n = 20)	Supraventricular tachycardia or atrial flutter (n = 18)
	Ventricular tachycardia (n = 2)
Bradyarrhythmia (n = 11)	Sinus bradycardia (n = 5)
	Second-degree atrioventricular block (n = 1)
	Complete atrioventricular block (n = 5)

CHD, congenital heart defect.
*Four fetuses had arrhythmia complicated by CHD; one fetus with supraventricular tachycardia had a cardiac tumor, and two fetuses with complete atrioventricular block and two fetuses with sinus bradycardia had left atrial isomerism.

comparable in control fetuses and those with CHD or arrhythmia.

3.2. Comparison of plasma NP levels in UA versus UV samples

Plasma ANP and BNP levels in maternal, UA, and UV samples are summarized in Table 3. In control fetuses, UV and UA plasma ANP levels were comparable ($p = 0.67$). Correlation analysis between UV and UA plasma ANP levels resulted in a regression coefficient and intercept of 0.49 and 17.66, respectively, indicating a significant correlation (r = 0.61, $p < 0.01$. Fig. 1A). In fetuses with CHD, UA plasma ANP levels were significantly higher than UV plasma ANP levels ($p < 0.01$). A significant correlation between UV and UA plasma ANP levels was observed, with a regression coefficient and intercept of 0.43 and 22.21, respectively (r = 0.71, $p < 0.01$. Fig. 1B). In fetuses with arrhythmia,

Table 3
Comparison of plasma ANP and BNP levels in maternal, UA, and UV samples.

	Control (n = 127)	CHD (n = 86)	Arrhythmia (n = 31)
Plasma ANP levels, pg/mL			
Maternal vein	62.2 ± 39.9‡	46.4 ± 30.5‡	53.8 ± 27.4‡
UA	38.6 ± 30.7	106.9 ± 122.5†	163.8 ± 273.2†
UV	36.1 ± 24.0	67.8 ± 75.1	116.2 ± 163.2
Decrease due to passing through the placenta*	2.1 ± 24.9	37.4 ± 87.1	44.2 ± 147.3
Plasma BNP levels, pg/mL			
Maternal vein	28.1 ± 26.4‡	16.9 ± 12.1‡	23.4 ± 14.6‡
UA	17.5 ± 11.5	61.1 ± 160.0	149.3 ± 281.9
UV	17.6 ± 10.6	49.5 ± 132.8	144.3 ± 270.9
Decrease due to passing through the placenta*	−0.3 ± 4.0	6.4 ± 44.3	0.6 ± 59.6

Data are shown as means ± SD.
ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHD, congenital heart defect; UA, umbilical artery; UV, umbilical vein.
*UA plasma NP levels – UV plasma NP levels.
†$p < 0.01$, Compared with UV.
‡$p < 0.05$, Compared with UV.

UA plasma ANP levels tended to be higher than UV plasma ANP levels ($p = 0.09$). A significant correlation between UV and UA plasma ANP levels was observed, with a regression coefficient and intercept of 0.54 and 31.69, respectively (r = 0.89, $p < 0.01$. Fig. 1C). Interestingly, in some cases the UV plasma ANP levels were higher than the UA plasma ANP levels in some cases in the control (61/127, 48.0%), CHD (33/86, 38.4%), and arrhythmia groups (12/31, 38.7%).

In control fetuses, UV and UA plasma BNP levels were comparable ($p = 0.52$). A significant correlation between UV and UA plasma BNP levels was observed, with a regression coefficient and intercept of 0.86 and 2.62, respectively (r = 0.93, $p < 0.01$. Fig. 1D). In fetuses with CHD, UV and UA plasma BNP levels were comparable ($p = 0.22$). A significant correlation between UV and UA plasma BNP levels was observed, with a regression coefficient and intercept of 0.85 and 2.15, respectively (r = 0.96, $p < 0.01$. Fig. 1E). In fetuses with arrhythmia, UV and UA plasma BNP levels were comparable ($p = 0.93$). A significant correlation between UV and UA plasma BNP levels was observed, with a regression coefficient and intercept of 0.95 and 6.61, respectively (r = 0.98, $p < 0.01$. Fig. 1F).

Collectively, the regression coefficient values between UV and UA

Table 2
Perinatal characteristics (n = 244).

	Control (n = 127)	CHD (n = 86)	Arrhythmia (n = 31)
Maternal age, years	33.4 ± 4.5	31.4 ± 5.4*	32.3 ± 4.7
Primipara status	48 (37.8)	39 (45.4)	21 (67.7)*
Latest cardiovascular profile score	10.0 ± 0	9.0 ± 1.7*	8.4 ± 2.2*
Latest biophysical profile score	10.0 ± 0.1	9.7 ± 1.0*	9.0 ± 1.1*
Polyhydramnios or oligohydramnios	1 (0.8)	7 (8.1)	4 (12.9)
Cesarean delivery	82 (64.6)	22 (25.6)*	18 (58.1)*
Gestational age at birth, weeks	38.0 ± 1.3	38.4 ± 1.6*	37.2 ± 1.4*†
Preterm birth	8 (6.3)	8 (9.3)	4 (12.9)
Birth weight, g	2912 ± 353	2835 ± 505	2621 ± 358*†
Small for gestational age (< 10th percentile)	0	20 (23.3)*	8 (25.8)*
Male infant	67 (53.2)	43 (50.0)	19 (61.3)
Neonatal death within 1 month	0	1 (1.2)	1 (3.2)
Infant death from 1 to 3 months	0	2 (2.3)	0
Apgar score ≤7 at 5 min	0	4 (4.7)	5 (16.1)*
Umbilical artery pH < 7.15	0	1 (1.2)	0
Ductal dependence	0	34 (39.5)*	1 (3.2)†

Data are shown as means ± SD or n (%).
CHD, congenital heart defect.
*$p < 0.05$, Compared with the control group.
†$p < 0.05$, Compared with the CHD group.

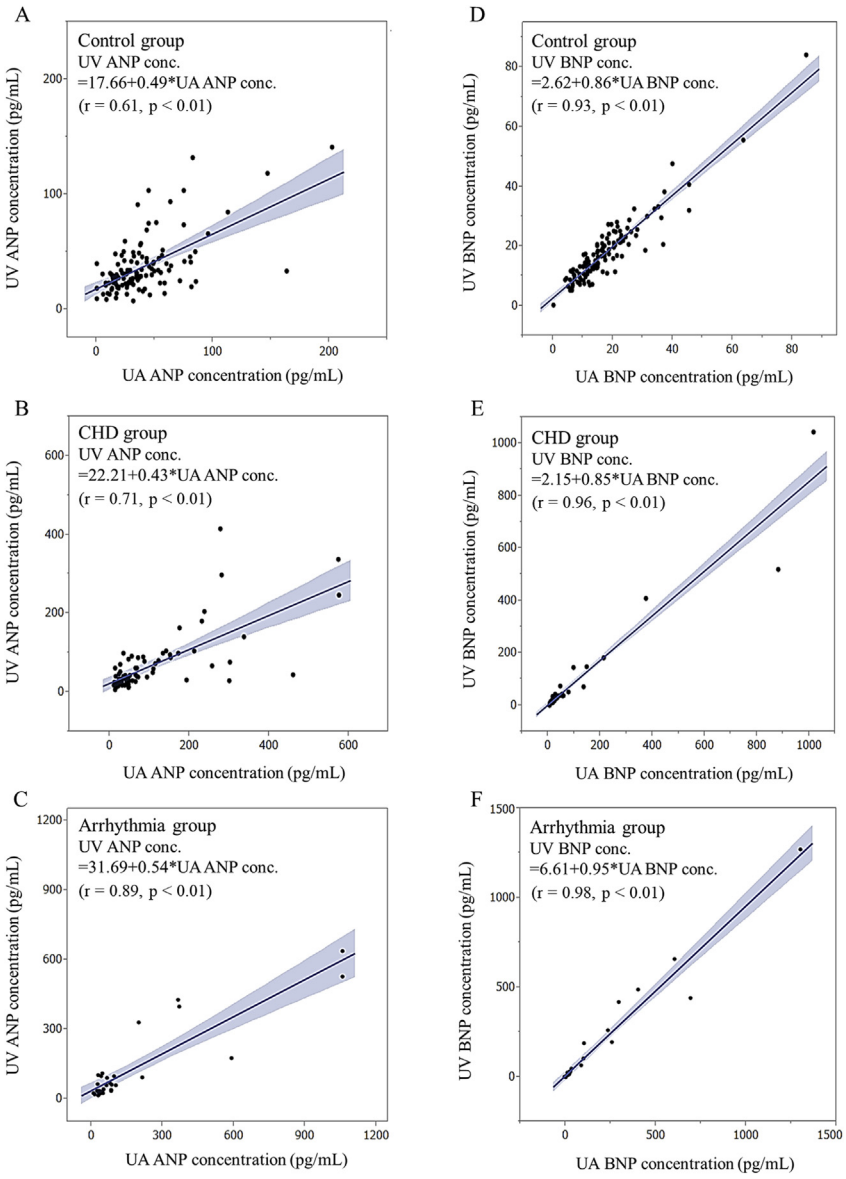

Fig. 1. Correlation analysis of plasma ANP and BNP concentrations in fetuses with CHD or arrhythmia and controls.Correlation analysis of plasma ANP and BNP levels in UV versus UA samples was performed using Pearson's coefficients.(A) In control fetuses, a significant correlation between UV and UA plasma ANP levels was observed, with a regression coefficient and intercept of 0.49 and 17.66, respectively (r = 0.61, $p < 0.01$).(B) In fetuses with CHD, a significant correlation between UV and UA plasma ANP levels was observed, with a regression coefficient and intercept of 0.43 and 22.21, respectively (r = 0.71, $p < 0.01$).(C) In fetuses with arrhythmia, a significant correlation between UV and UA plasma ANP levels was observed, with a regression coefficient and intercept of 0.54 and 31.69, respectively (r = 0.89, $p < 0.01$).(D) In control fetuses, a significant correlation between UV and UA plasma BNP levels was observed, with a regression coefficient and intercept of 0.86 and 2.62, respectively (r = 0.93, $p < 0.01$).(E) In fetuses with CHD, a significant correlation between UV and UA plasma BNP levels was observed, with a regression coefficient and intercept of 0.85 and 2.15, respectively (r = 0.96, $p < 0.01$) (F) In fetuses with arrhythmia, a significant correlation between UV and UA plasma BNP levels was observed, with a regression coefficient and intercept of 0.95 and 6.61, respectively (r = 0.98, p < 0.01).ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHD, congenital heart defect; UA, umbilical artery; UV, umbilical vein; conc., concentration (pg/mL).

plasma ANP levels were close to 0.5, while those between UV and UA plasma BNP levels were close to 1. The intercept values between UV and UA plasma ANP levels were over 17, while those between UV and UA plasma BNP levels were close to 0. These findings in the plasma ANP and BNP levels were consistent across fetus groups (CHD, arrhythmia, and controls), indicating common metabolic features of each NP.

3.3. Comparison of NP levels in fetuses and mothers

In controls, UV plasma ANP levels were significantly lower than maternal plasma ANP levels ($p < 0.01$), and UV plasma BNP levels were significantly lower than maternal plasma BNP levels ($p < 0.01$). In fetuses with CHD, UV plasma ANP levels were significantly higher than maternal plasma ANP levels ($p = 0.01$), and UV plasma BNP levels were significantly higher than maternal plasma BNP levels ($p = 0.03$). In fetuses with arrhythmia, UV plasma ANP levels were significantly higher than maternal plasma ANP levels ($p = 0.02$), and UV plasma BNP levels were significantly higher than maternal plasma BNP levels ($p = 0.02$). In controls and fetuses with CHD or arrhythmia, no correlation was found between fetuses and mothers in terms of either plasma ANP or BNP levels.

3.4. RP-HPLC analysis of UA plasma in controls and fetuses with CHD or arrhythmia

The molecular forms of immunoreactive ANP in UA plasma showed a single, 28-residue peak corresponding to mature alpha-ANP, the endogenous biologically active form (Fig. 2A–C). No difference in the proportion of ANP molecular forms was found between controls and fetuses with CHD or arrhythmia. The molecular forms of immunoreactive BNP in UA plasma showed two major peaks and several minor peaks, consisting of mature BNP (BNP-32, with 32 residues), glyco-proBNP, and proBNP (Fig. 2D–F). The mature and precursor forms of BNP were the major fractions responsible for BNP immunoreactivity in UA plasma; however, the proportion of BNP molecular forms differed among individual controls and fetuses with CHD or arrhythmia.

3.5. RT-PCR analysis of normal human placental tissues

Gene expressions of *NPR1* and *NPR3* were confirmed in placental tissue samples from two healthy women (Fig. 3). This suggested the presence of NPR-A and NPR-C in the human placenta.

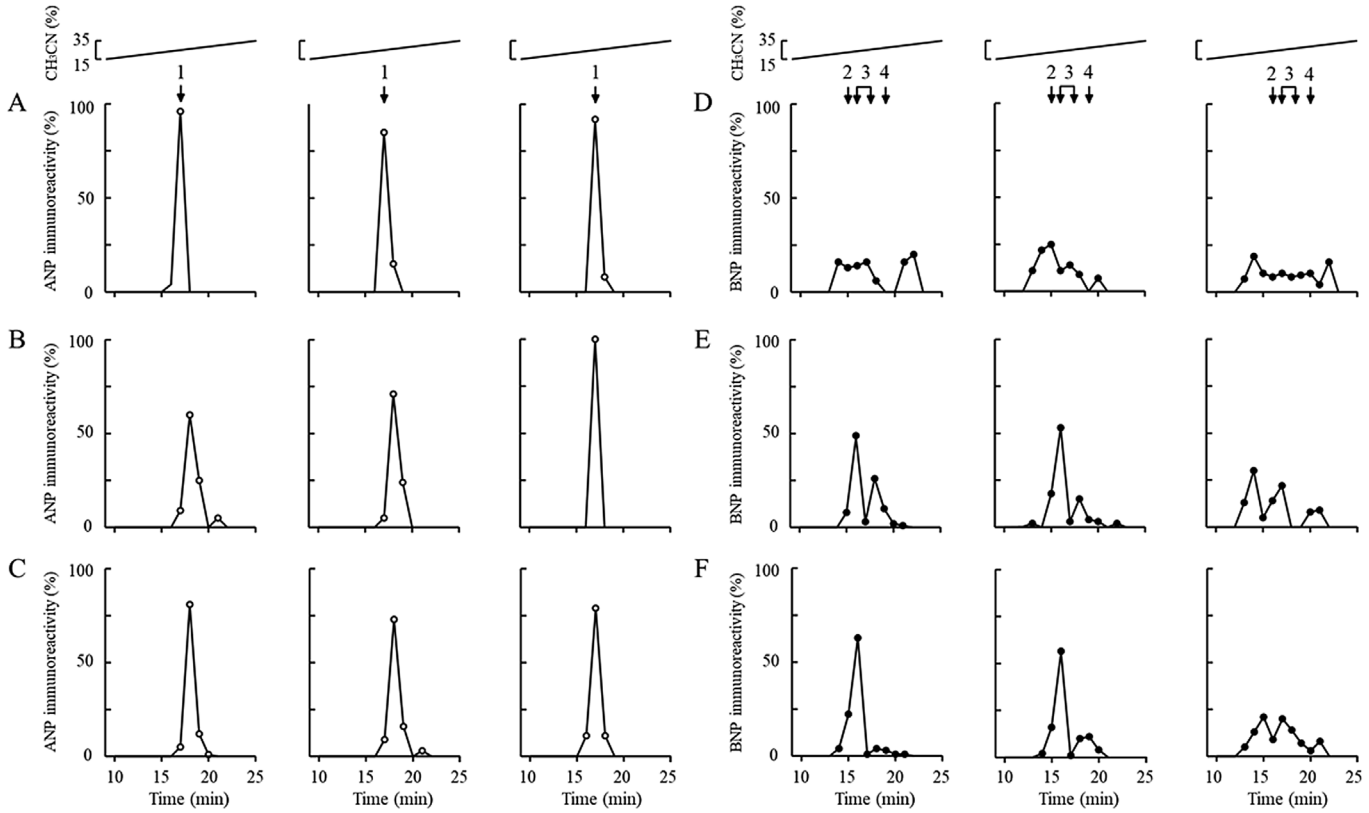

Fig. 2. RP-HPLC analysis of UA plasma in fetuses with CHD or arrhythmia and controls The molecular forms of immunoreactive ANP in UA plasma were characterized by an AIA-PACK chemiluminescence immunoassay coupled with RP-HPLC in control fetuses (A) and fetuses with CHD (B) or arrhythmia (C). Peak 1 is the elution time of mature ANP (alpha-ANP, 28 amino acids), an endogenous fully active form. The molecular forms of immunoreactive BNP in UA plasma were also characterized by AIA-PACK chemiluminescence immunoassay coupled with RP-HPLC in control fetuses (D) and fetuses with CHD (E) or arrhythmia (F). Peaks 2, 3, and 4 indicate elution times of mature BNP (BNP-32, 32 amino acids), glyco-proBNP, and proBNP, respectively. Glyco-proBNP demonstrates a broader peak than the others on RP-HPLC due to its heterogenous properties. Two major peaks and several minor peaks are observed. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHD, congenital heart defect; glyco, glycosylated; proBNP, BNP precursor; RP-HPLC, reverse-phase high-performance liquid chromatography; UA, umbilical artery.

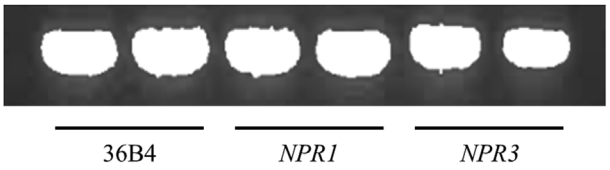

Fig. 3. RT-PCR analysis of normal human placental tissues (n = 2).Gene expressions of *NPR1* and *NPR3*, with 36B4 as a reference, were confirmed in placental tissue samples from two healthy women.NPR, natriuretic peptide receptor; RT-PCR, reverse transcription polymerase chain reaction.

4. Discussion

The principal finding of our study was the differential metabolism of ANP and BNP in the fetoplacental circulation. Plasma ANP levels in UV and UA samples were strongly correlated, and the same was true for plasma BNP levels. The regression coefficient between UV and UA plasma ANP levels was close to 0.5, while that between UV and UA plasma BNP levels was close to 1, suggesting that the placental and umbilical vessels may be major sites of ANP metabolism but not of BNP metabolism. In addition, we found that circulating ANP consisted of its mature form, whereas circulating BNP consisted predominantly of its precursor forms. The different proportions of molecular forms may lead to the differences in metabolism between ANP and BNP that were observed in the fetoplacental circulation.

For the first time, we demonstrated a difference between ANP and BNP metabolism in the fetoplacental circulation. After passing through the placenta, ANP levels in UV plasma decreased to approximately one-half the levels in UA plasma in fetuses with CHD or arrhythmia and in controls, suggesting that the placenta and umbilical vessels may be major sites of ANP metabolism. Conversely, plasma BNP levels barely decreased after passing through the placenta, regardless of the type or presence of fetal heart disease. It has been reported that BNP in human plasma has an approximately 10-fold longer half-life than ANP [11]. Two pathways are known to clear circulating NPs: binding and internalization via NPR-C, and enzymatic degradation by peptidase [3,4]. NPR-C-mediated degradation is the major mechanism responsible for the clearance of NPs from the circulation [2]. The binding affinity of ANP for NPR-C is greater than that of BNP in both humans and rats [12,13]. A major peptidase responsible for NP degradation is neutral endopeptidase (NEP), also known as neprilysin; also involved is a secondary peptidase, namely insulin-degrading enzyme, and numerous other minor peptidases [2]. Human plasma BNP degradation is delayed by the addition of BNP, which is rather resistant to NEP [14]. Walther et al. investigated the role of NEP in the turnover of plasma ANP and BNP in fetuses with Rhesus isoimmunization, and showed that in contrast to ANP metabolism, BNP degradation was not dependent on NEP [15]. Therefore, the lower affinity of BNP for NPR-C and its resistance to NEP digestion may result in BNP being more stable in the fetoplacental circulation.

RP-HPLC analyses in this study revealed that in the fetoplacental circulation, ANP consisted of the mature form, while BNP consisted predominantly of the precursor forms, regardless of differences in the type or presence of fetal heart disease. Recent publications have shown

that the glycosylated precursor, glyco-proBNP, is a major circulating component in adults with heart failure, a result of impairment of processing events by the glycosylation of threonine-71 of proBNP [16–19]. ProBNP is highly glycosylated and has properties that differ from those of the simple 108-residue proBNP peptide. The presence of circulating highly glycosylated proBNP may help prevent BNP from being metabolized in the placenta and umbilical vessels, since glycosylation generally affords protection against proteases [20]. These differences in the circulating molecular forms are also responsible for different properties in the metabolic clearance capabilities of each NP.

Commercially available immunoreactive BNP assays overrepresent the biological activity of the NP system because they cannot distinguish between active and inactive forms [2,21]. Liang et al. reported that the cyclic guanosine-3′,5′-monophosphate (cGMP)-producing activity of proBNP in cultured endothelial cells and vascular smooth muscle cells was only about one-eighth to one-sixth that of mature BNP [17]. The potency of cGMP production via NPR-A is greater for mature ANP than for mature BNP [12,22]. Interestingly, previous reports demonstrated that ANP, but not BNP, is expressed in the human placenta, particularly in cytotrophoblast cells [23,24]. In this study cohort, there were several cases in which ANP levels were higher in UV than UA plasma, supporting the possibility that ANP is secreted from the placenta locally or into the fetoplacental circulation. Taken together, ANP may play a pivotal role in the regulation of fetoplacental hemodynamics.

In controls and fetuses with CHD or arrhythmia, no correlation was found between fetuses and mothers in terms of either plasma ANP or BNP levels. The available evidence indicates that the human placenta is permeable to peptide hormones [25], but transplacental transfer of BNP is considered to be negligible [26,27]. Moreover, our previous study demonstrated that umbilical cord plasma NP levels were correlated with the severity of heart failure in fetuses with CHD or arrhythmia [6]. No differences in NP levels were found between fetuses without heart failure and controls. Collectively, it is probable that the mother and fetus secrete ANP and BNP independently of each other, and that the fetal heart is a main origin of elevated NP levels in the umbilical cord plasma of fetuses with CHD or arrhythmia. Therefore, plasma concentrations of ANP and BNP in the fetoplacental circulation are likely to be regulated by the balance between production by the fetal heart and metabolism and clearance in the placenta and umbilical vessels.

There were several limitations in the present study. First, since this was a sub-analysis of our previous study [6], placental tissue samples were not collected for the analysis of gene and protein expression levels. Therefore, commercially available placental tissue samples were used to perform gene expression analyses of *NPR1* and *NPR3* to confirm the presence of these mRNAs. However, it is also essential to investigate the plasma concentration of each endogenous ANP and BNP molecular form in the fetoplacental circulation, as well as their binding affinity for NP receptors in the placental tissue of fetuses with CHD or arrhythmia and controls. This is because the expression levels of these peptides and their receptors might be affected by the pathophysiology as well as the severity of fetal heart failure. Second, we were not able to investigate the relationship between molecular forms of NPs and mortality after birth, because of rather low neonatal and infant mortality in the present cohort. A recent study showed that in adult patients with acute decompensated heart failure, compensation for heart failure occurred via increased proBNP processing, leading to an increase of mature BNP and activation of the BNP/cGMP cascade [28]. In the future, large-scale studies are needed to clarify the prognostic value of different ANP and BNP molecular forms in fetuses with heart failure.

In conclusion, the fetus and mother are considered to independently produce and metabolize ANP and BNP. Metabolism in the fetoplacental circulation is quite different between ANP and BNP. Fetal plasma ANP consists of the mature form, and the placenta and umbilical vessels may be major sites of ANP metabolism. In contrast, fetal plasma BNP consists predominantly of the precursor forms, which may contribute to preventing BNP from being metabolized in the placenta and umbilical vessels.

Conflicts of interest

The authors have no competing interests to declare.

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Author contributions

HH contributed to the conception and design of this work and serves as the corresponding author. TM evaluated fetal echocardiographic findings in all cases and collected umbilical cord blood samples and clinical data. TM contributed to the statistical analysis and wrote the manuscript. TM and JY diagnosed and managed cases with CHD and arrhythmia. HH, MM, and NM measured ANP and BNP concentrations. HH performed RT-PCR and RP-HPLC analysis. HH, KK and NM compiled, revised, and proofread the manuscript.

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